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United States Patent [19]**Regnier et al.**[11] **Patent Number:** **5,958,202**[45] **Date of Patent:** **Sep. 28, 1999**[54] **CAPILLARY ELECTROPHORESIS ENZYME IMMUNOASSAY**WO 95/20161 7/1995 WIPO .
WO 97/22825 6/1997 WIPO .[75] Inventors: **Fred E. Regnier**, West Lafayette, Ind.;
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Framingham, Mass.[21] Appl. No.: **08/792,933**[22] Filed: **Jan. 22, 1997****Related U.S. Application Data**

[63] Continuation-in-part of application No. 08/386,224, Feb. 9, 1995, Pat. No. 5,810,985, which is a continuation of application No. 07/944,846, Sep. 14, 1992, abandoned.

[51] **Int. Cl.**⁶ **G01N 27/26**; G01N 33/53;
G01N 33/561; C12Q 1/68[52] **U.S. Cl.** **204/451**; 204/601; 435/6;
435/7.7; 435/7.92; 436/516[58] **Field of Search** 204/451, 455,
204/452, 461, 601, 605, 603, 612, 606;
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516[56] **References Cited****U.S. PATENT DOCUMENTS**4,181,589 1/1980 Brooks 435/173.9
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Primary Examiner—Robert Warden*Assistant Examiner*—Alex Noguerola*Attorney, Agent, or Firm*—Testa, Hurwitz & Thibault, LLP[57] **ABSTRACT**

Disclosed are methods and apparatus for performing highly sensitive enzyme-amplified assays using channel electrophoresis of an enzyme having a biorecognition moiety. Combining a binding reaction of an analyte and an enzyme having a biorecognition moiety, and an electrophoretic separation which uses enzyme-amplified detection methodology, assay methods and apparatus are provided that offer simplicity, quantitative sensitivity and rapid analysis. Also disclosed are highly sensitive catalytically-amplified assays using a capillary electrophoresis format in which the binding reaction prior to electrophoresis is heterogeneous or homogeneous, and direct or competitive. The methods and apparatus for conducting sensitive automated catalytically-amplified assays using electrophoresis may be conducted in a microscale format.

29 Claims, 23 Drawing Sheets